ADAPTATION OF ATOMIC ABSORPTION EQUIPMENT FOR PYRIDINE NUCLEOTIDE ASSAYS AND ENZYMATIC ANALYSES

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The atomic absorption spectrophotometer conventionally has a selection of hollow cathode lamps for different elements, a flame-atomiser system, a monochromator and a detector with read-out. By a simple interchange of the burner for a cuvette holder it becomes useful for absorption measurements at those wavelengths at which the lamps emit sufficient energy. The rhodium lamp* has a line at 343.5 nm and the chromium lamp* has one at 357.9 nm. Either wavelength is suitable for absorption measurements on solutions of reduced pyridine nucleotide (either NADH or NADPH) but the specific absorption at the lower wavelength is about 1.5 times higher than at the longer wavelength. With either model of the Techtron (Techtron Pty. Ltd., Melbourne, Australia) instrument it is simple to arrange a cuvette holder in the optical path at the focal plane between lamp and slit. For the smaller AA100 a dummy plug to fit the burner socket supports a platform on which a standard sliding cuvette holder, which may be thermostated, is mounted. For the AA4 a cell holder is fitted to a slide on the optical bench. Quantities of 50 nmoles of NAD(P)H upwards can be measured directly in 2.07 ml buffer by use of the optical density scale, on the readout 50 nmoles corresponds to 0.1 O.D. change. With the existing expanded scale fed to a recorder the accuracy is about 10% (see fig. 1). The limitations are: (1) noise from the lamp, and (2) slow drift. The drift can be reduced by feeding the instrument through a constant voltage transformer and in any case it can be measured and allowed for by taking readings through a blank cuvette. The instrument can be used for all those enzymatic assays which depend on oxidation or reduction of a pyridine nucleotide. The progress is followed using the recorded readout.

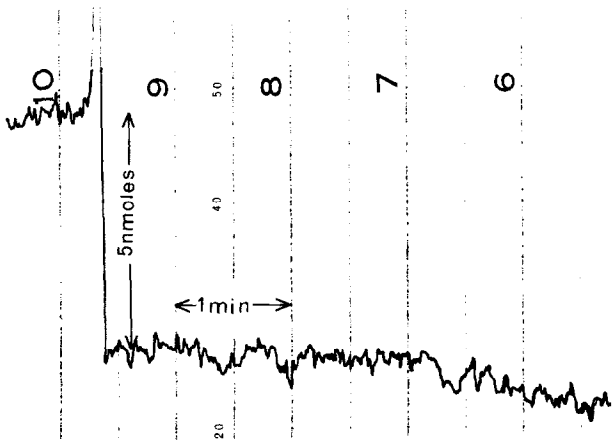

Fig. 1. The displacement on the expanded scale chart obtained by adding 5 nmoles NADH to a volume of 2 ml in the 1 cm pathlength cuvette. The chromium lamp was used as source and at this wavelength (357.9 nm) 5 nmoles in 2 ml correspond to an optical density of 0.01 units.

The intensity of the emission in the Techtron instrument is modulated at 50 or 70 cps and an alternating current amplifier is used in the detector. For this reason the cuvette is used fully exposed to daylight which increases the ease of making successive additions to it. The low energy used in the system makes it unsuitable for measurements on turbid solutions which attenuate the beam.

* The chromium (Type HCN-Cr) and rhodium (Type HCN-Rh) lamps were supplied by Varian Assoc. Molesey Rd. Walton-on-Thames, Surrey, UK.